First Three Neisseria gonorrhoeae Isolates with High-Level Resistance to Azithromycin in Sweden: a Threat to Currently Available Dual-Antimicrobial Regimens for Treatment of Gonorrhea?

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Neisseria gonorrhoeae has developed resistance to all antimicrobials previously used for first-line treatment of gonorrhoea (1). Treatment failures with the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone, the only remaining antimicrobials for monotherapy in most countries, have now emerged in several countries (2–5). Consequently, in the United States (6) and Europe (7), dual-antimicrobial therapies recommending ceftriaxone (250 to 500 mg [one dose]) together with azithromycin (1 to 2 g [one dose]) have been introduced. Recently evaluated novel dual-antimicrobial regimens also include azithromycin (together with gentamicin or gemifloxacin) (8). Moreover, in the United States, azithromycin (2 g, one dose) is recommended if the patient has a severe cephalosporin allergy (6), and despite not being recommended, in several countries, azithromycin (1 to 2 g [one dose]) monotherapy remains to be used. It is of grave concern that during recent years, rare gonococcal strains with high-level azithromycin resistance (MIC ≥ 256 µg/ml) have been identified in the United Kingdom, Italy, Argentina, and the United States (2).

This study describes the first three gonococcal isolates with high-level resistance to azithromycin in Sweden (identified in 2011 to 2012). The gonococcal isolates were cultured from urethral specimens from three patients with symptomatic urethritis, and the species was verified with a sugar utilization test and the Phadebact multiantigen sequence type (NG-MAST) and sequencing of the 23S rRNA gene. The isolates were assigned to NG-MAST ST285 and the novel ST8727 (Table 1).

The gonorrhea cases and the corresponding gonococcal isolates are described in Table 1. The azithromycin MIC for all isolates was 4,096 µg/ml. The mean ΣFICs for the combination of azithromycin and ceftriaxone for the three isolates were 0.66 (additive effect), 1.05 (indifferent effect), and 1.47 (indifferent effect). All isolates had an mtrR resistance determinant that results in an overexpressed MtrCDE efflux pump and accordingly increased MICs of azithromycin as well as of ESCs (2, 10, 11) and, most importantly, an A2059G transition in three to four alleles of the 23S rRNA gene. The isolates were assigned to NG-MAST ST285 and the novel ST8727 (Table 1).

This study describes the first three gonococcal isolates with high-level azithromycin resistance in Sweden, and for the first time, we determine the azithromycin endpoint MIC (4,096 µg/ml) for gonococcal isolates with the A2059G transition in three to four of the 23S rRNA alleles, previously associated with high-level resistance to azithromycin (2). Fortunately, the mean ΣFICs showed that no antagonism existed; instead, an additive or indifferent effect existed for the combination of azithromycin and ceftriaxone. Nevertheless, for all isolates, the ceftriaxone MICs were clearly elevated (0.032 to 0.064 µg/ml), and if those isolates acquire an appropriate penA mosaic allele, they will become resistant to current dual-antimicrobial regimens. The isolates were cultured from three patients residing in two Swedish cities, and all patients were infected in their home city. The epidemiological characterization showed that the isolates represented two gonococcal strains, and high-level azithromycin resistance has not previously been reported for isolates of the identified STs, ST285 and ST8727.

### Table 1: Characteristics of the first Neisseria gonorrhoeae isolates with high-level resistance to azithromycin identified in Sweden

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Yr of isolation</th>
<th>Gender of patient, age (yr)</th>
<th>City of residence</th>
<th>MIC (µg/ml) (susceptibility category) a,b</th>
<th>ΣFICs</th>
<th>Alleles of the 23S rRNA gene with a mutation c</th>
<th>NG-MAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2011</td>
<td>Male, 25</td>
<td>Jonkoping</td>
<td>Azithromycin 4,096 (R)</td>
<td></td>
<td>1, 2, and 4</td>
<td>ST285</td>
</tr>
<tr>
<td>2</td>
<td>2012</td>
<td>Male, 49</td>
<td>Karlstad</td>
<td>Ceftriaxone 0.064 (S)</td>
<td></td>
<td>1, 2, 3, and 4</td>
<td>ST8727</td>
</tr>
<tr>
<td>3</td>
<td>2012</td>
<td>Female, 46</td>
<td>Karlstad</td>
<td>Cefixime 0.032 (S)</td>
<td></td>
<td>1, 2, 3, and 4</td>
<td>ST8727</td>
</tr>
</tbody>
</table>

Notes:
- a High-level resistance was indicated by a MIC of ≥256 µg/liter. All patients had symptomatic urethritis. The serovars were Bropyst, Bropys, and Bropys for patients 1, 2, and 3, respectively.
- b NG-MAST, N. gonorrhoeae multiantigen sequence type.
- c S, susceptible; I, intermediate susceptible; R, resistant. Determinations of susceptibility were in accordance with the breakpoints stated by the Clinical and Laboratory Standards Institute (CLSI; www.clsi.org) or, for azithromycin, for which the CLSI has not stated any resistance breakpoint, the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org).
- d An A2059G transition in domain V of the 23S rRNA alleles results in decreased target affinity and, if present in several of the four alleles, in high-level azithromycin resistance.
Subsequently acquired epidemiological information verified that the ST8727 isolates were cultured from two patients with sexual contact. All patients were successfully treated with 400 mg cefixime (one dose).

In conclusion, the international spread of several gonococcal clones with high-level azithromycin resistance is of grave concern and threatens the long-term sustainability of available dual-antimicrobial regimens for gonorrhea treatment. Replacement of azithromycin with the new fluoroketolide solithromycin (12, 13) in the current dual-antimicrobial regimens might be at least a short-term solution. Ultimately, novel antimicrobials for antimicrobial monotherapy and/or dual therapy for gonorrhea are essential.

REFERENCES